



# **Glive ekstremnih okolij: adaptacije, tveganja in uporabnost**

Cene Gostinčar

Ektremofilne / ekstremotolerantne glive:

**KDO SO?**



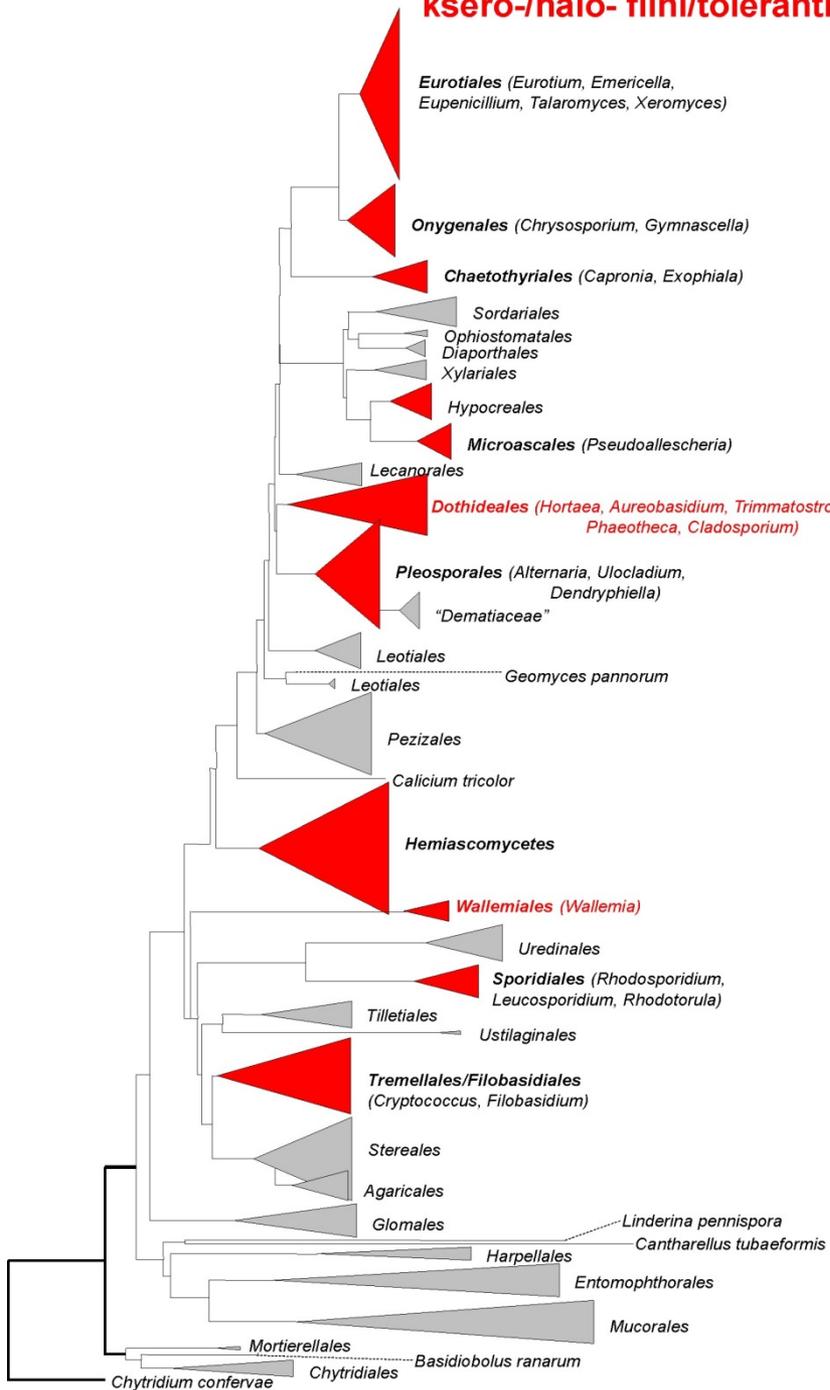


Gunde-Cimerman N., Zalar P., de Hoog S., Plemenitaš A. 2000. **Hypersaline waters in salterns - natural ecological niches for halophilic black yeasts.** FEMS Microbiology, Ecology, 32, 3: 235-240.



Butinar L., Spencer-Martins I., Gunde-Cimerman N. 2007. **Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms.** Antonie van Leeuwenhoek, 91, 3: 277-289.

# ksero-/halo- filni/tolerantni predstavniki



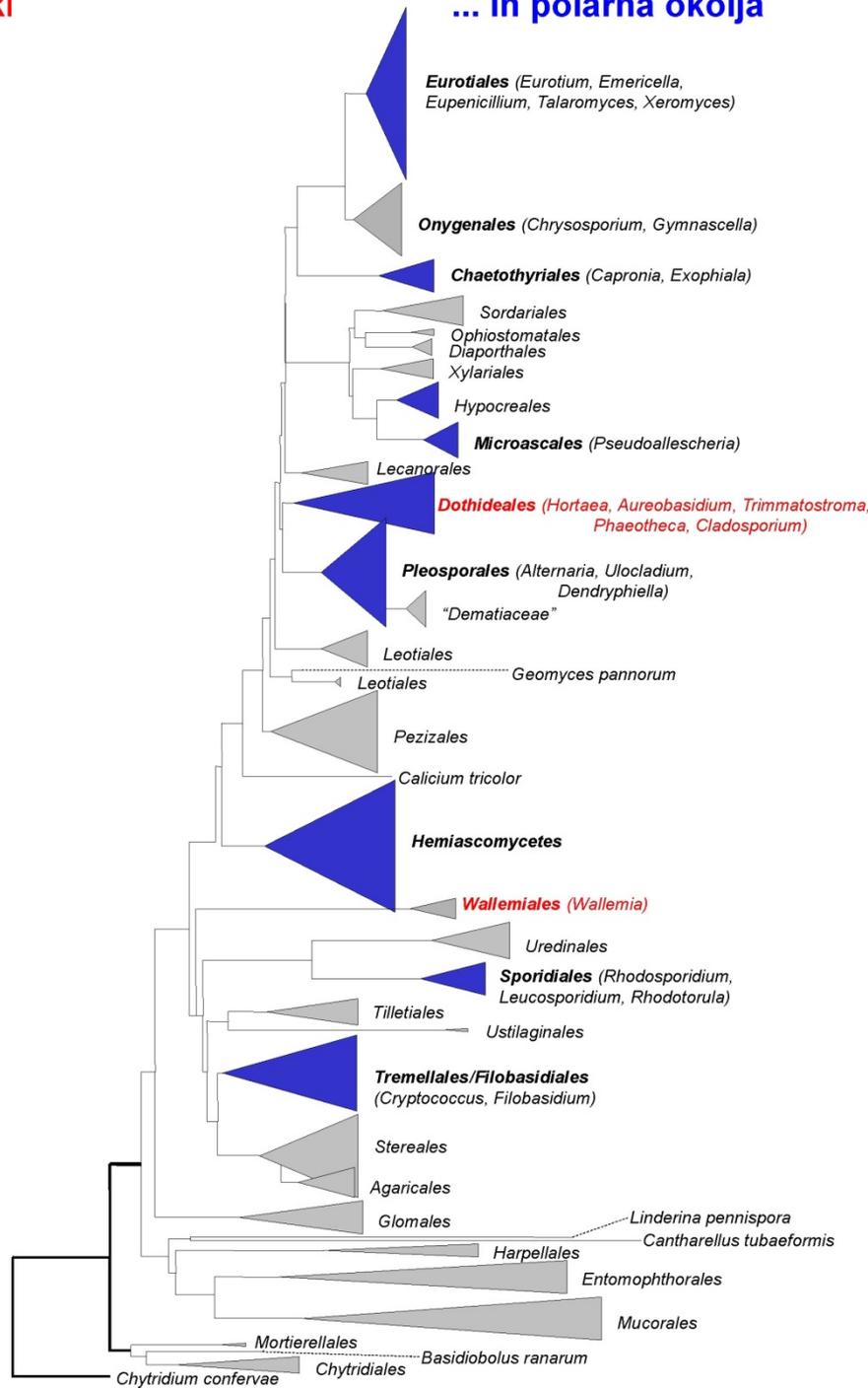
## Ascomycota

## Basidiomycota

## Zygomycota

## Chytridiomycota

# ... in polarna okolja



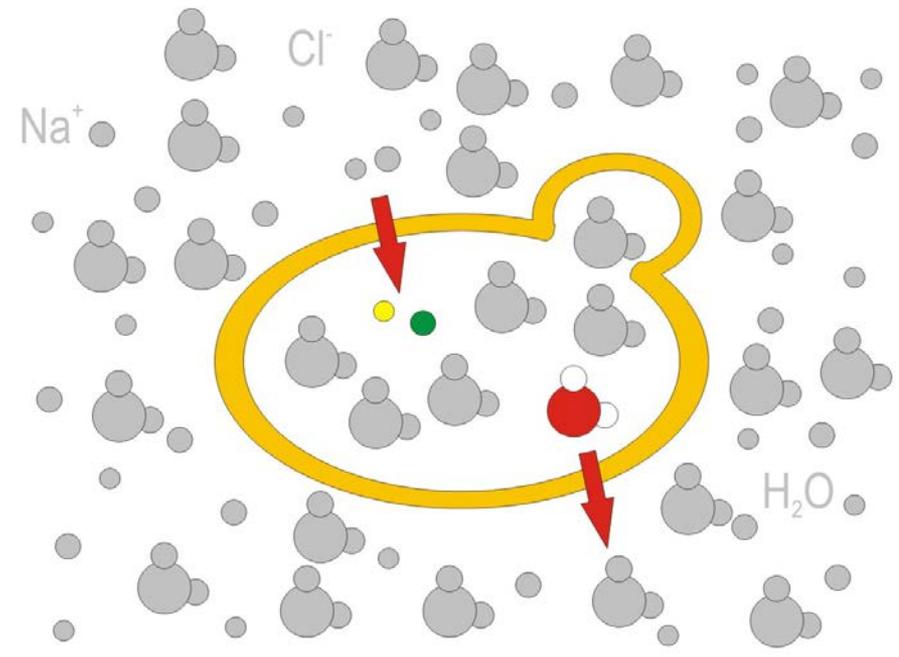
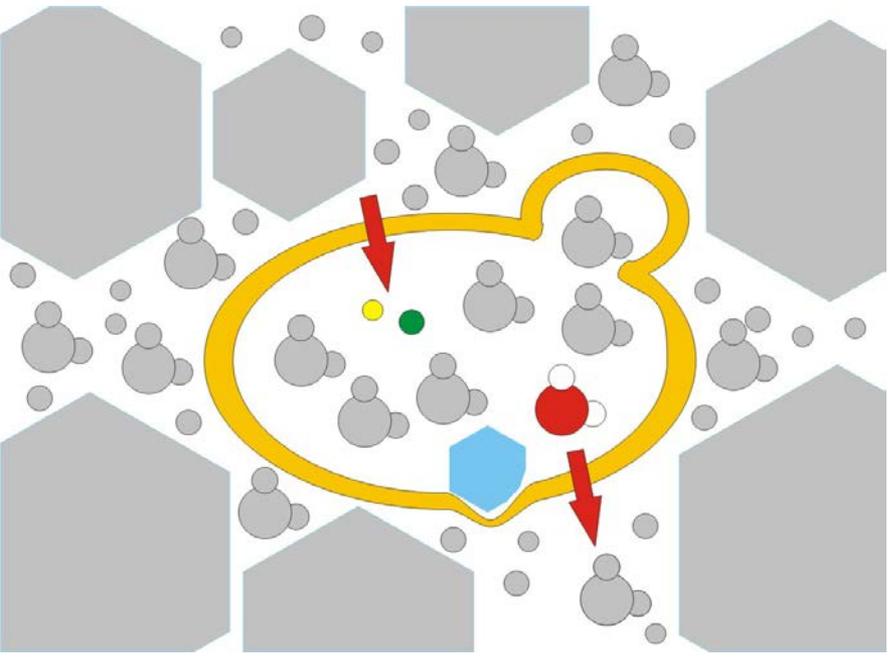
Ekstremofilne/ekstremotolerantne glive živijo  
v razmerah, v katerih propade večina ostalih organizmov:

**KAKO PREŽIVIJO?**





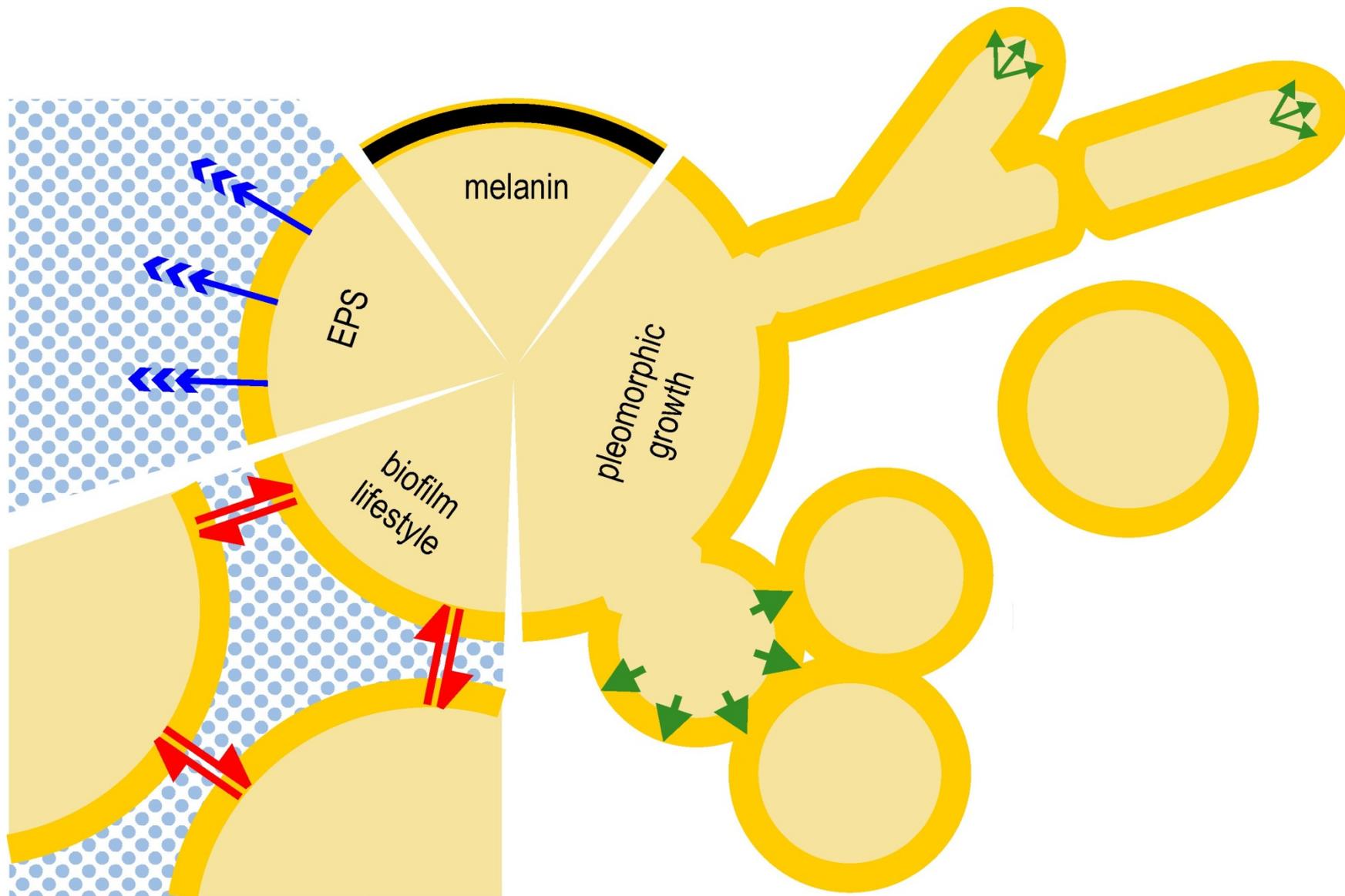
Led: pomanjkanje vode, kristali ledu, visoke koncentracije anorg. ionov.



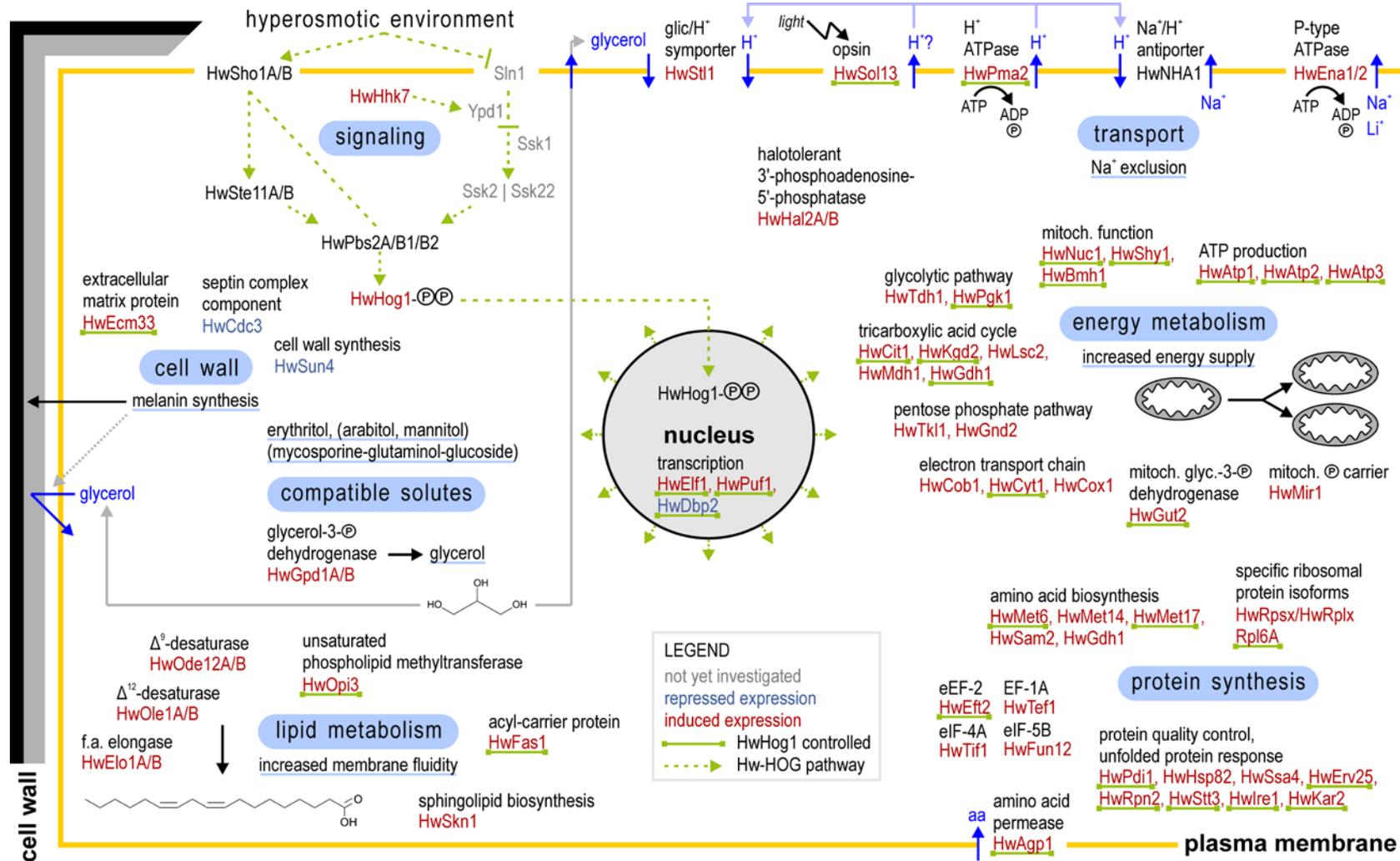
Soline: pomanjkanje vode, visoke koncentracije anorganskih ionov.



# Splošni mehanizmi tolerance na stres: primer poliekstremotolerantnih črnih gliv

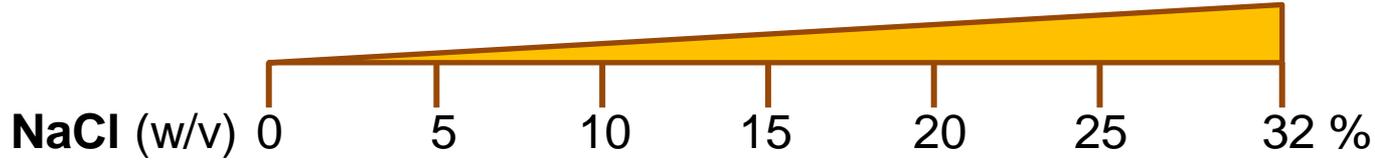


# Specializirane prilagoditve: primer črne kvasovke *Hortaea wernecki*



Gostinčar C, Lenassi M, Gunde-Cimerman N, Plemenitaš A: Fungal Adaptation to Extremely High Salt Concentrations. Adv Appl Microbiol 2011, 77:71-96.

# Tri glive: tri strategije preživetja.



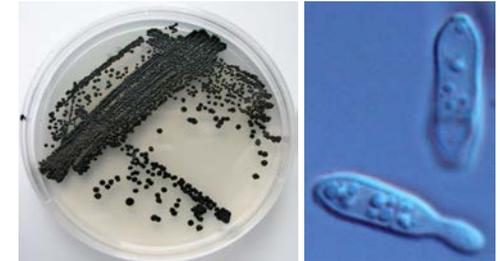
halotoleranca

*Aureobasidium pullulans*



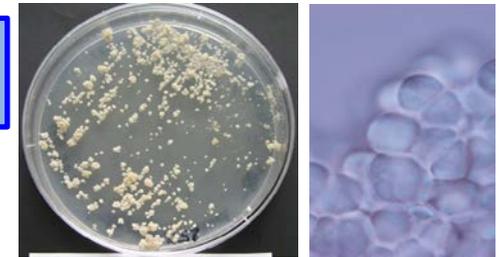
ekstremna halotoleranca

*Hortaea werneckii*

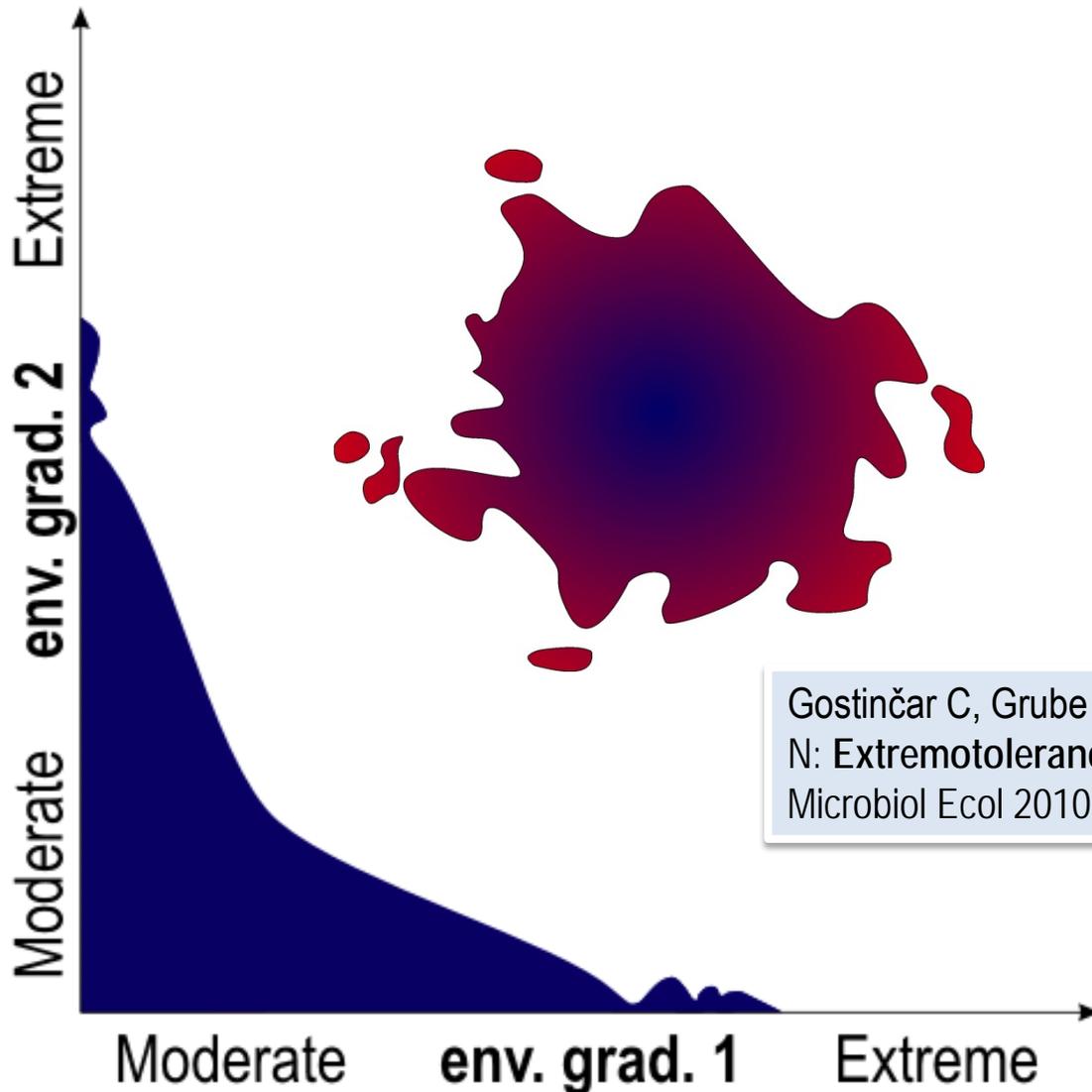


halofilija

*Walleimia ichthyophaga*



# Evolucija: 1. Preživetje

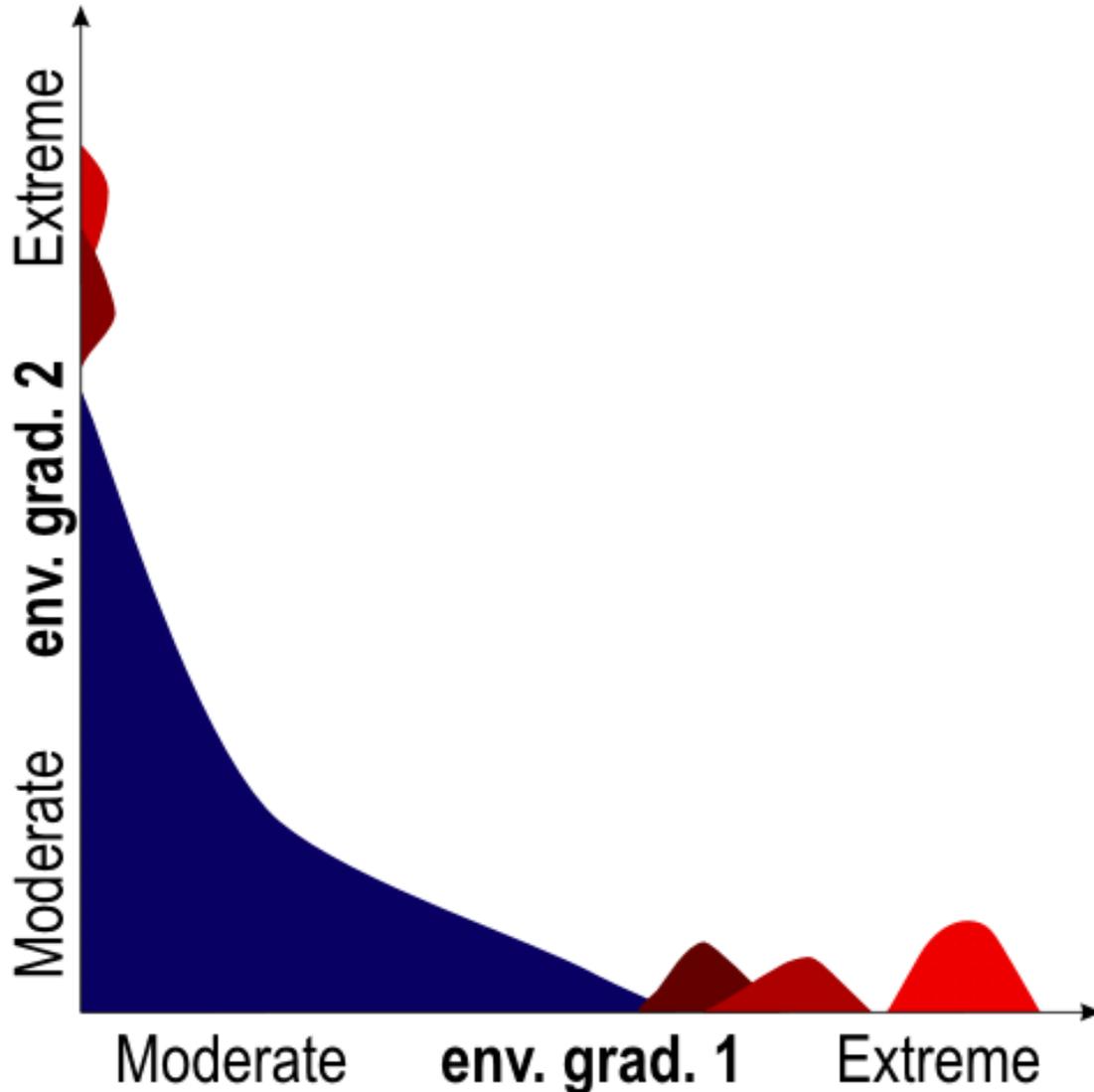


ekstremne razmere na robu  
fragmentacija habitatov  
genski drs, ozka grla  
povečana hitrost mutacij?  
ekološko prileganje

**Zelo hitro!**

Gostinčar C, Grube M, de Hoog GS, Zalar P, Gunde-Cimerman N: Extremotolerance in fungi: evolution on the edge. FEMS Microbiol Ecol 2010, 71:2-11.

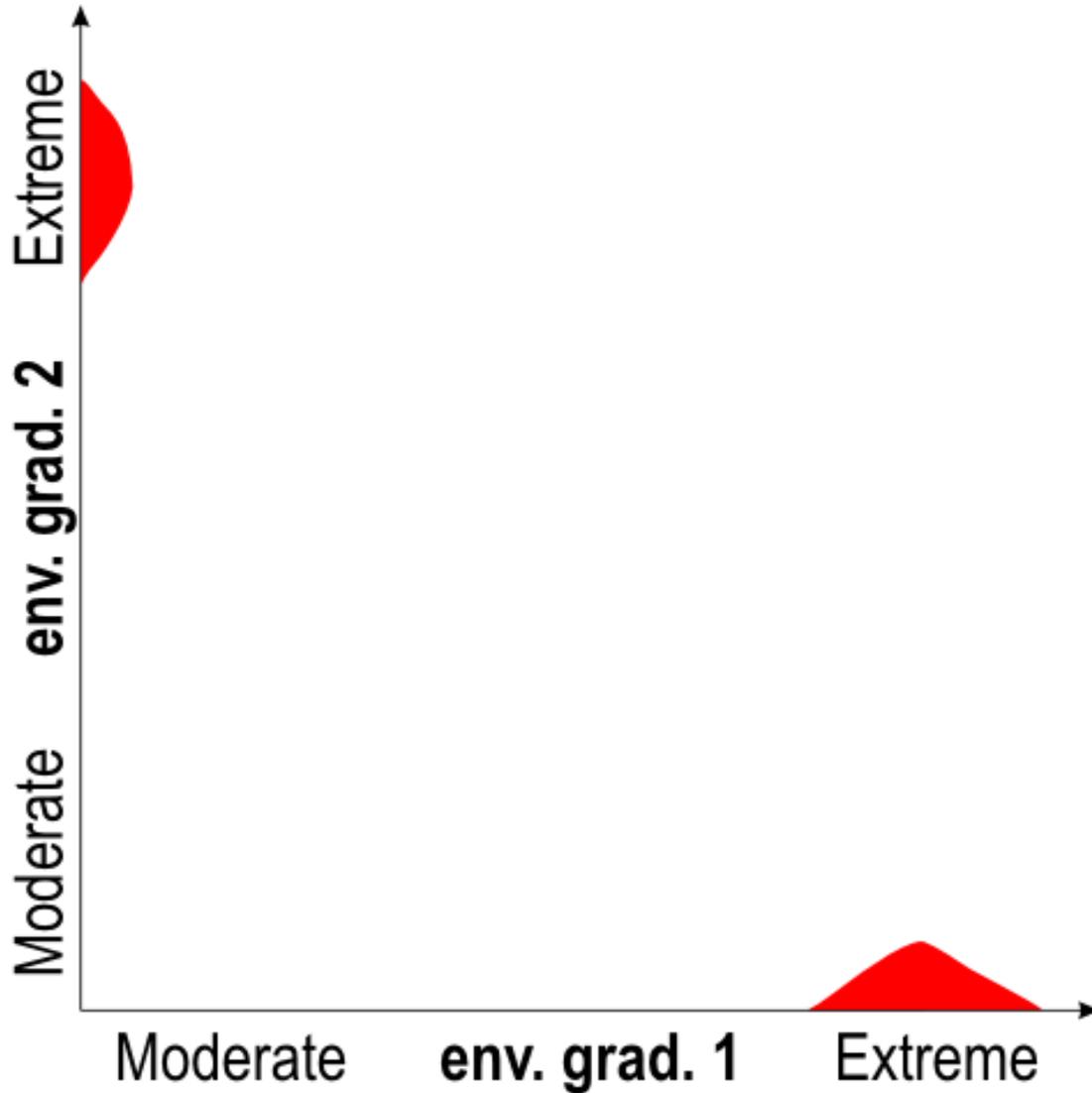
# Evolucija: 1. Adaptivna radiacija



prekinitev genskega pretoka  
(boljše ohranjanje adaptacij)

Zmerno hitra.

# Evolucija: 1. Specializacija



premik ravnega optima

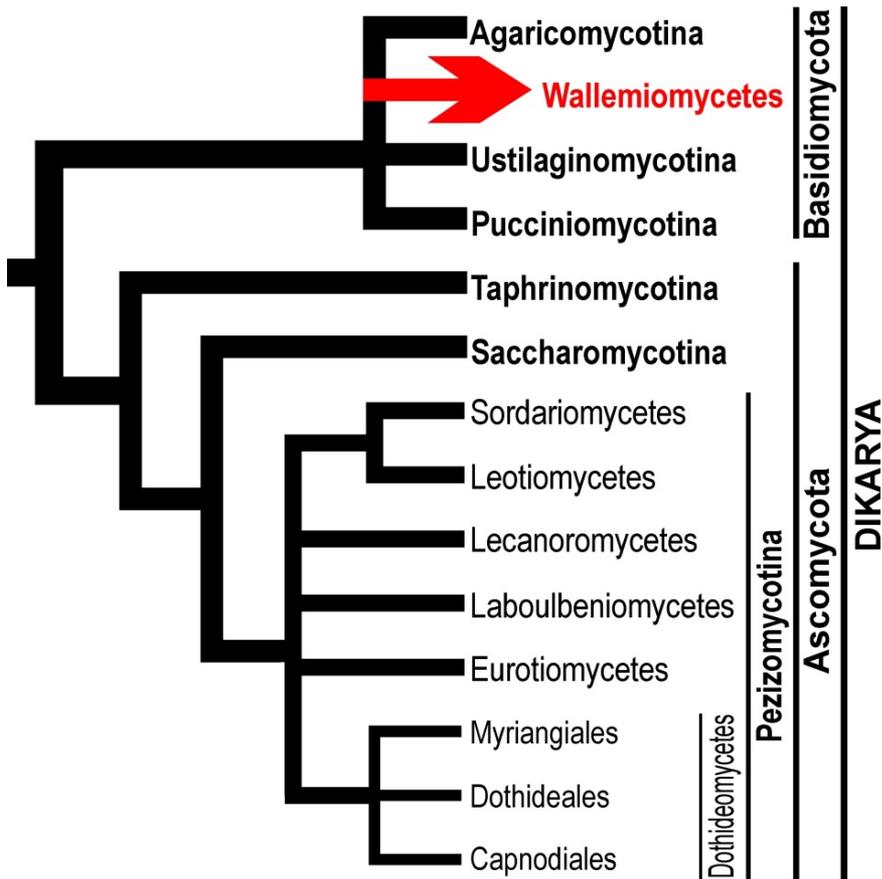
**Počasna.**

Novo obdobje v preučevanju gliv iz ekstremnih okolij:

# GENOMIKA

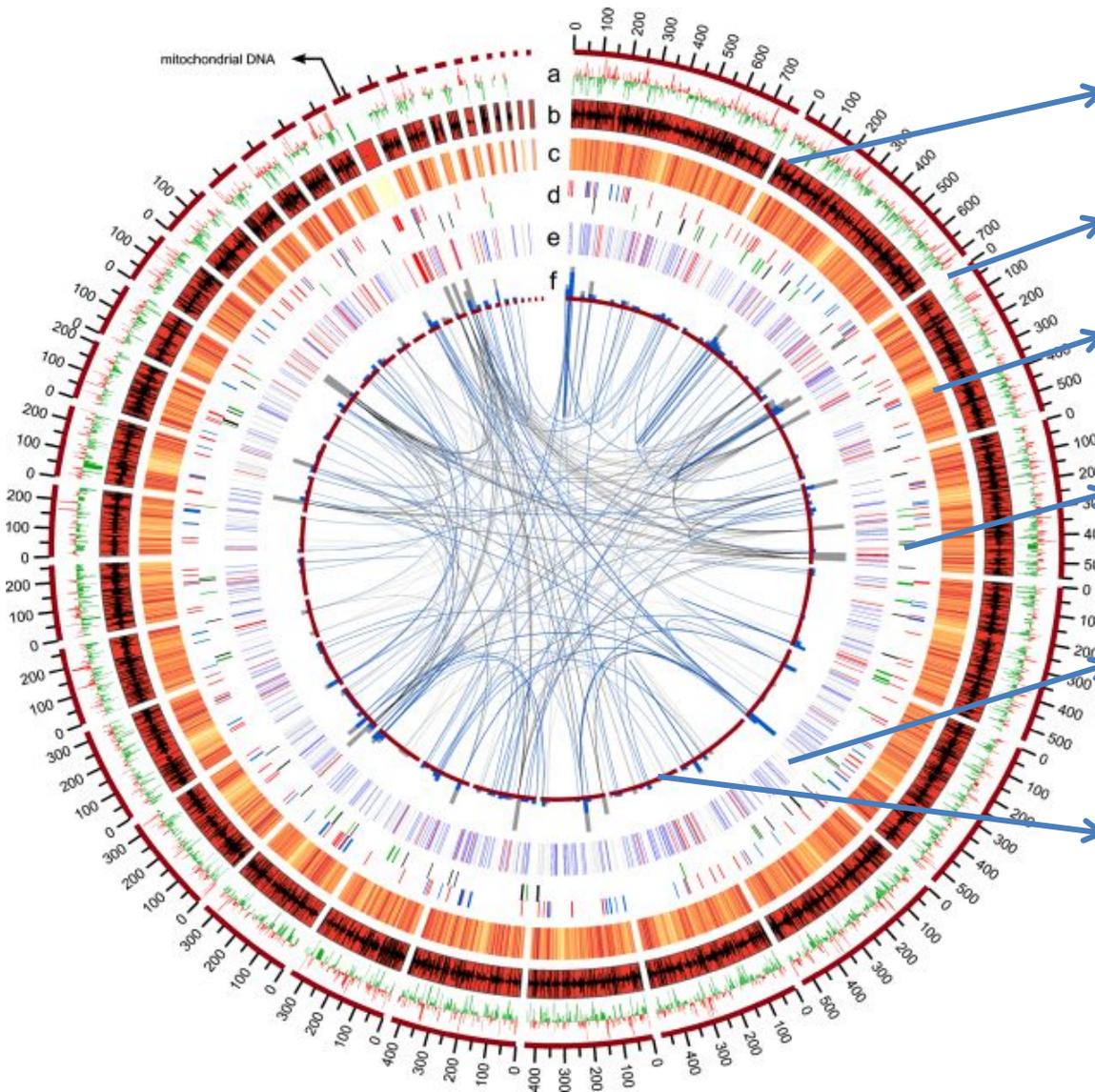


# Rod *Wallemia*: stara filogenetska linija prostotrosnic (Basidiomycota).



Janja Zajc\*, Yongfeng Liu\*, Wenkui Dai, Zhenyu Yang, Jinzhi Hu, Cene Gostinčar\*, Nina Gunde-Cimerman\*. Haloadaptations revealed by genome and transcriptome sequencing of the halophilic fungus *Wallemia ichtyophaga*. BMC Genomics 2013, 14:617.

# Izjemno majhen in kompakten genom.



**Sizes of scaffolds** >10 kbp, with a histogram showing the gene expression

**Differential expression** at 30% versus 10% NaCl [w/v]

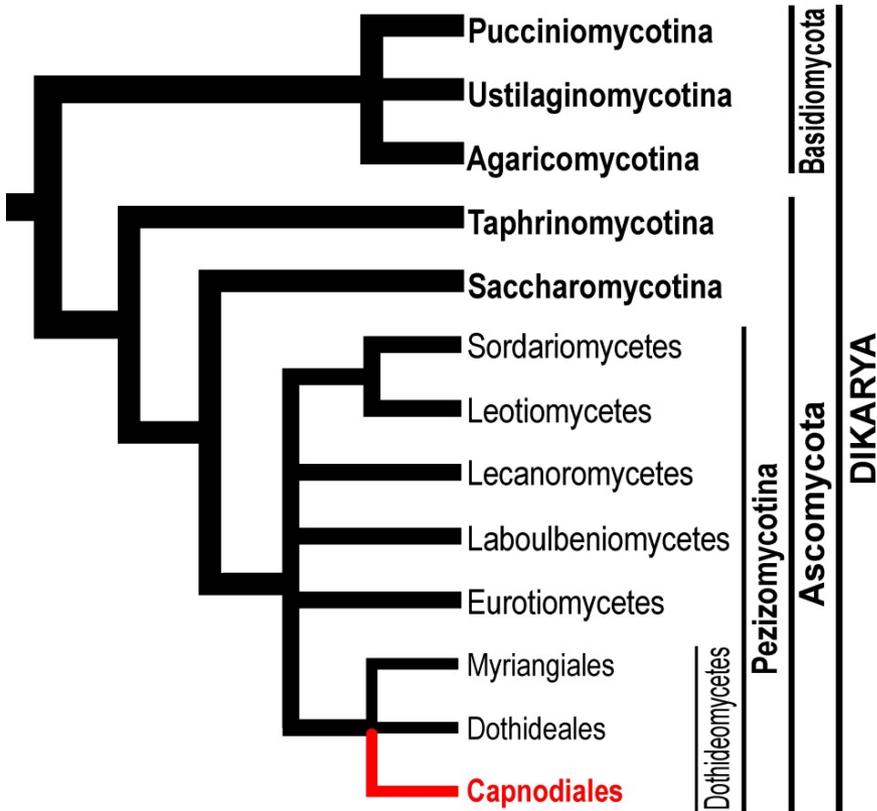
**GC content** on a scale from 30% (yellow) to 60% (red).

**Locations of certain groups of genes** (red, energy production; blue, cell cycle; black, cell wall; green, membrane transporters).

**Locations of repetitive sequences** (grey, tandem repeats; blue, transposons; red tRNA).

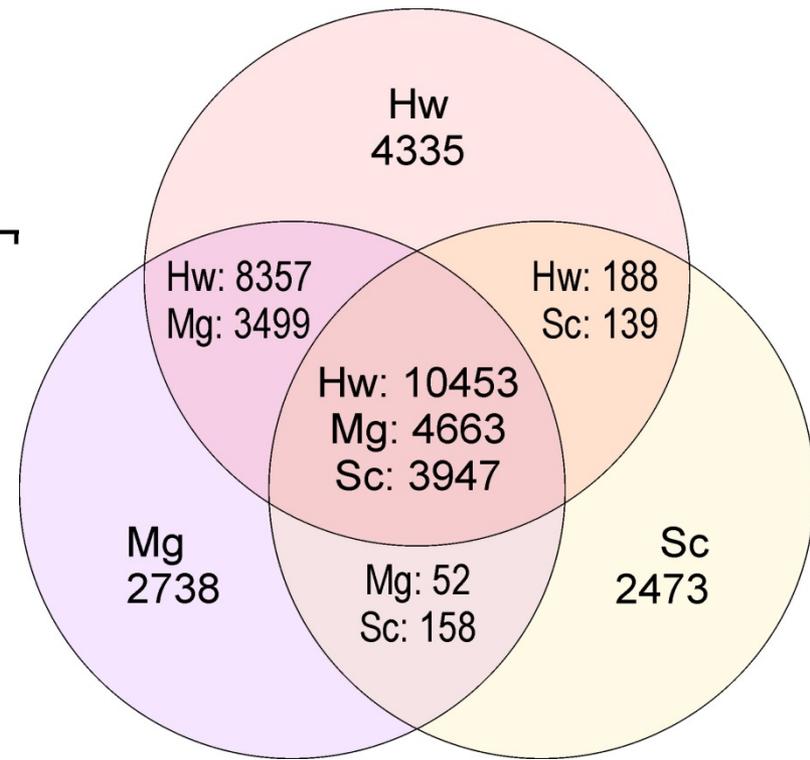
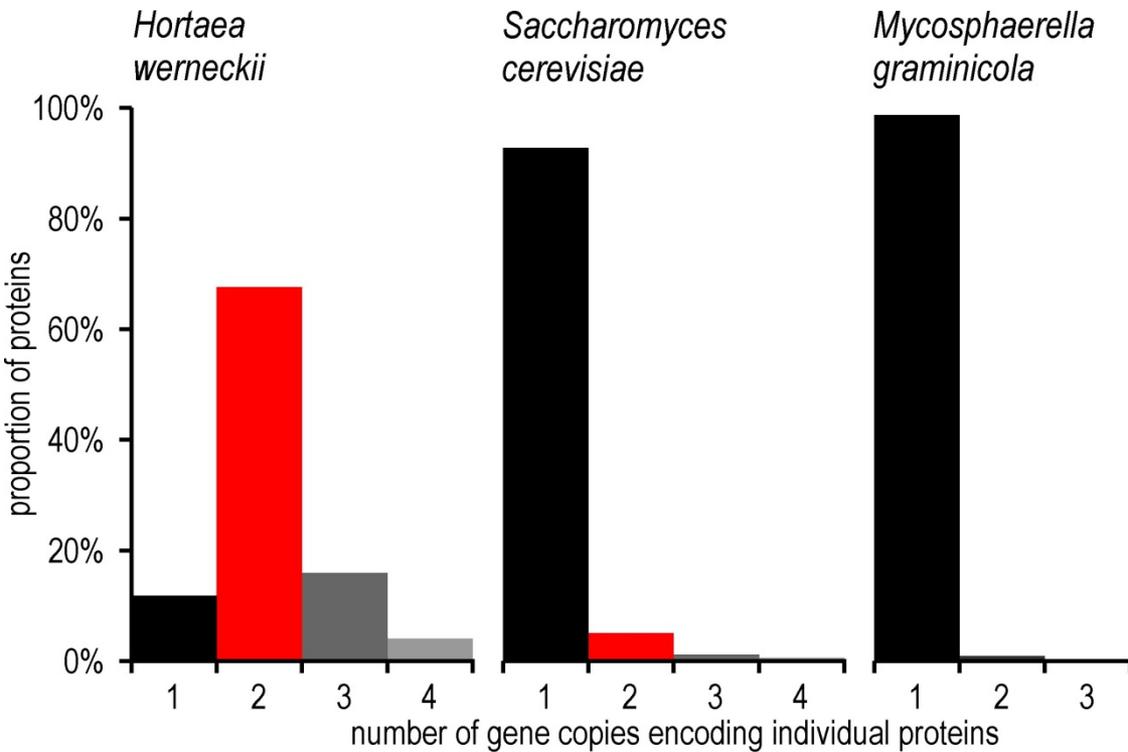
**Gene duplications and links** linking their locations to the genome (blue, proteins that aligned with more than 200 amino acids; grey, proteins that aligned with 100-200 amino acids).

# *Hortaea werneckii* – ekstremno halotolerantna črna kvasovka



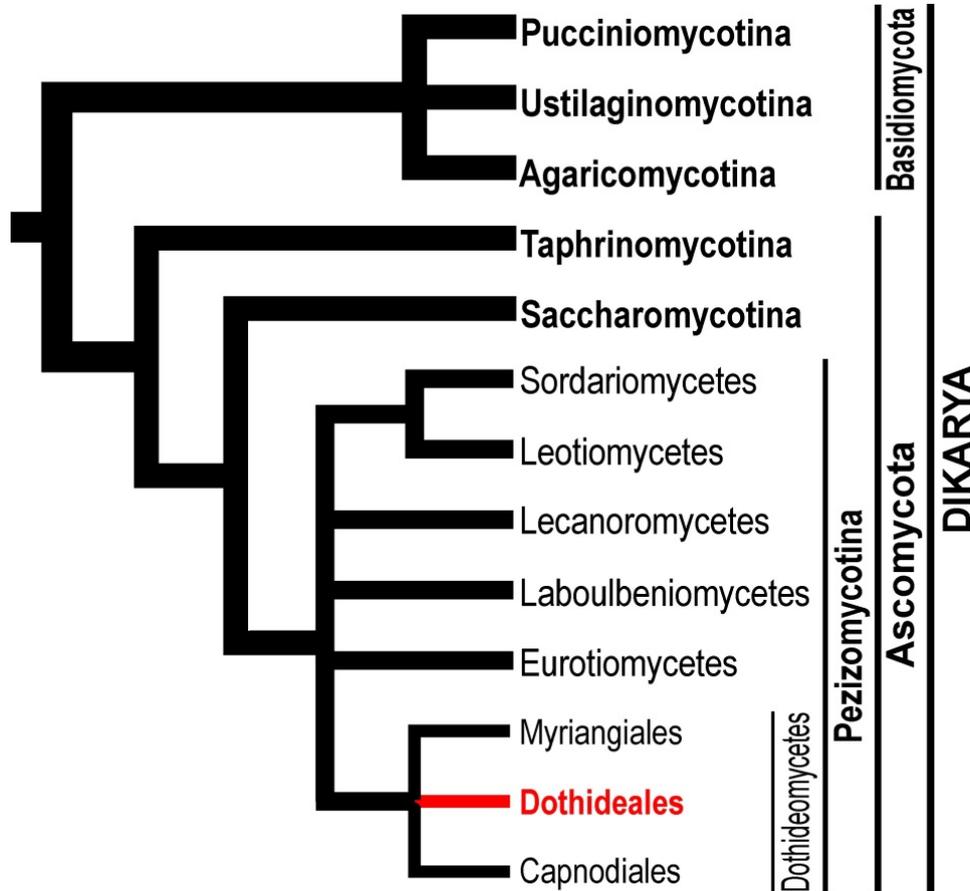
Metka Lenassi\*, Cene Gostinčar\*, Shaun Jackman, Martina Turk, Ivan Sadowski, Corey Nislow, Steven Jones, Inanc Birol, Nina Gunde Cimerman, Ana Plemenitaš. **Whole Genome Duplication and Enrichment of Metal Cation Transporters Revealed by de novo Genome Sequencing of Extremely Halotolerant Black Yeast *Hortaea werneckii*.** PLoS ONE 2013, 8:e71328.

# Duplikacija celotnega genoma.



# *Aureobasidium pullulans*:

poliekstremotolerantna črna kvasovka



Cene Gostinčar, Robin A. Ohm, Tina Kogej, Silva Sonjak, Martina Turk, Janja Zajc, Polona Zalar, Martin Grube, Hui Sun, James Han, Aditi Sharma, Jennifer Chiniquy, Chew Yee Ngan, Anna Lipzen, Kerrie Barry, Igor V. Grigoriev, Nina Gunde-Cimerman. Genome sequencing of four *Aureobasidium pullulans* varieties: biotechnological potential, stress tolerance, and description of new species. poslano v objavo

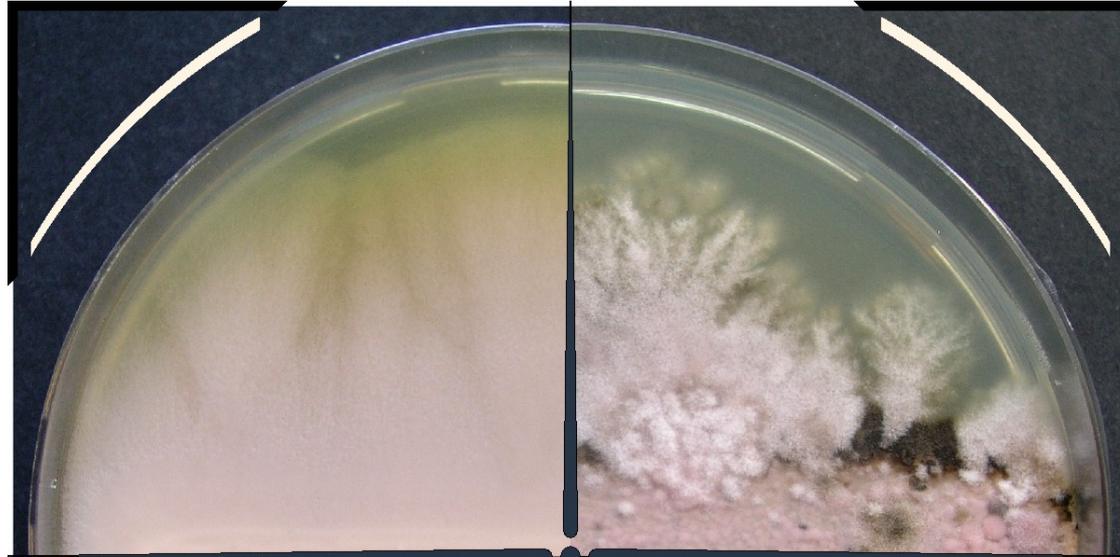
# Varietete naseljujejo različna okolja in rastejo v nekoliko različnih pogojih

15% NaCl

4/25/30°C



*var. pullulans*



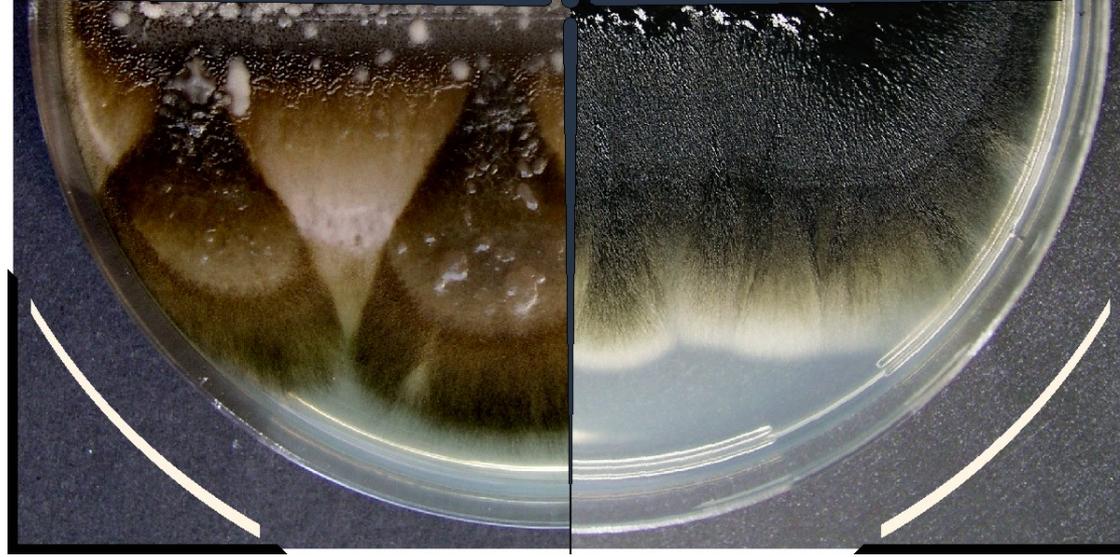
*var. subglaciale*

10% NaCl

4/25/25°C



*var. namibiae*



*var. melanogenum*

10% NaCl

10/25/30°C



10% NaCl

10/30/35°C



# Poliekstremotolerantna gliva: gliva multipraktik :)

**najdena v številnih okoljih:** - površina rastlin, ledeniki  
solinska voda, onesnažena voda, oligotrofna jezera  
- hrana: shranjena na hladnem, soljena, sušena  
- hišni prah, kopalnice, pomivalni stroji, vodovodna voda  
- rezervoarji za letalsko gorivo, razkrajajoča se plastika

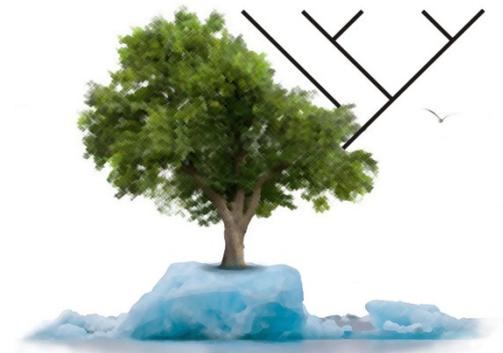
**tolerira številne strese:** visoke koncentracije soli, kislina in  
bazična okolja, nizke temperature, pomanjkanje hranil,  
sevanje

**varietete ► vrste:** *A. pullulans*, *A. melanogenum*, *A. subglaciale* in *A. namibiae*

**velika diverziteta številnih skupin genov:** membranski transporterji anorganskih ionov in sladkorjev, akvaporini, zunajcelični encimi

# Genomska statistika:

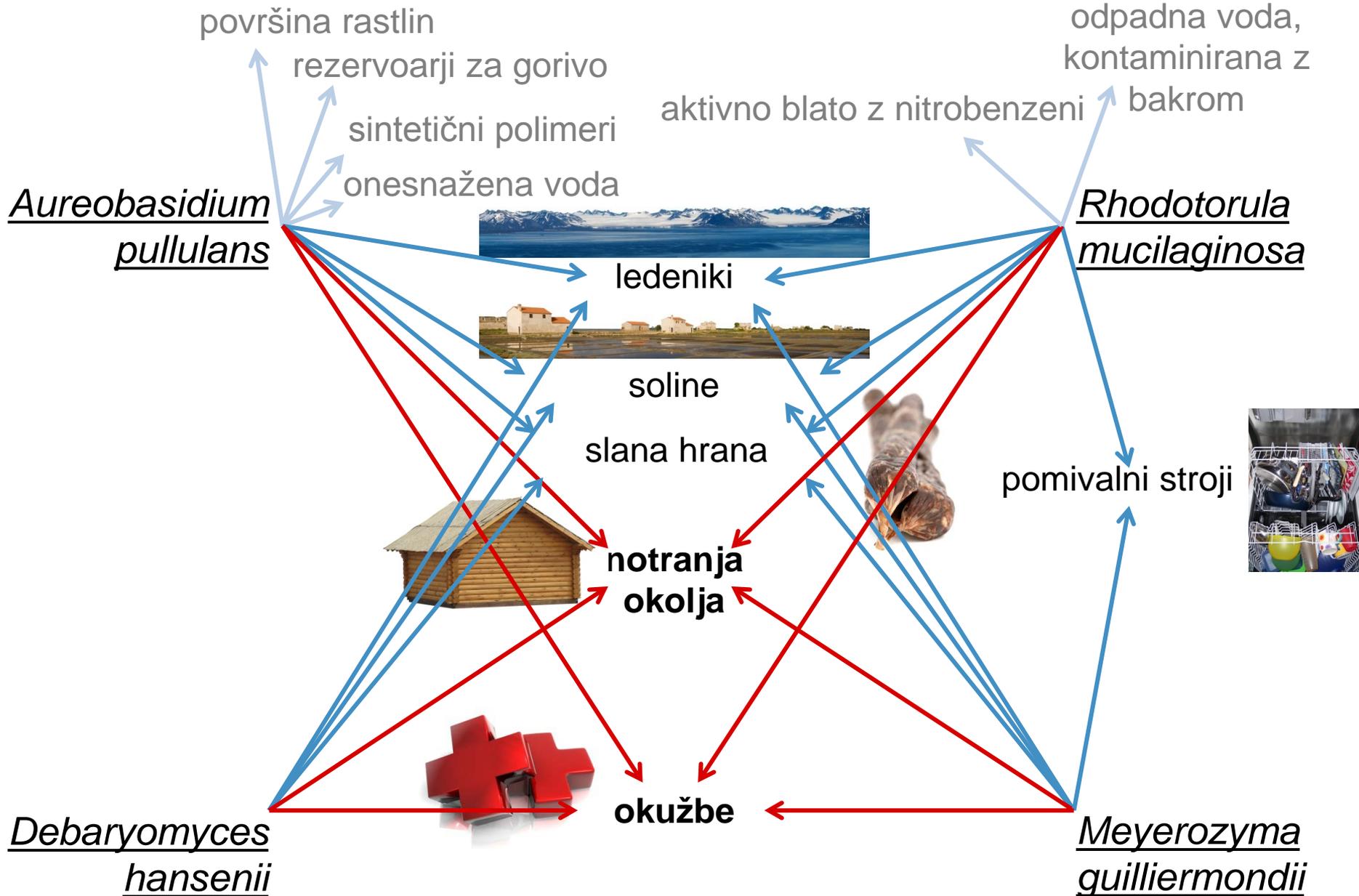
	<b>Wi</b>	<b>Hw</b>	<b>ApP</b>	<b>ApM</b>	<b>ApS</b>	<b>ApN</b>
Coverage	>270x	~ 70x	172.5x	140.2x	814x	138.7x
<b>Genome assembly size (Mbp)</b>	<b>9.63</b>	<b>51.6</b>	<b>29.62</b>	<b>26.20</b>	<b>25.80</b>	<b>25.43</b>
Number of contigs	101	~ 20 000	209	174	84	55
Number of scaffolds	82		186	150	75	47
<b>Gene models (n)</b>	<b>4884</b>	<b>23333</b>	<b>11866</b>	<b>10594</b>	<b>10809</b>	<b>10266</b>
Gene average lenght (bp)	1597		1629	1458	1643	1487
Exon average length (bp)	425		609	525	611	524
Exons per gene (average)	3.41		2.84	2.54	2.51	2.59
Intron average length (bp)	61		81	83	73	83
<b>GC content (%)</b>	<b>45.40%</b>	<b>54%</b>	<b>50.02%</b>	<b>49.85%</b>	<b>50.78%</b>	<b>51.12%</b>
GC content of exons (%)	47.51%	56%	52.52%	51.87%	52.53%	52.99%
<b>Repeat content (%)</b>	<b>1.67%</b>	<b>1.02%</b>	<b>1.45%</b>	<b>0.97%</b>	<b>0.87%</b>	<b>0.78%</b>



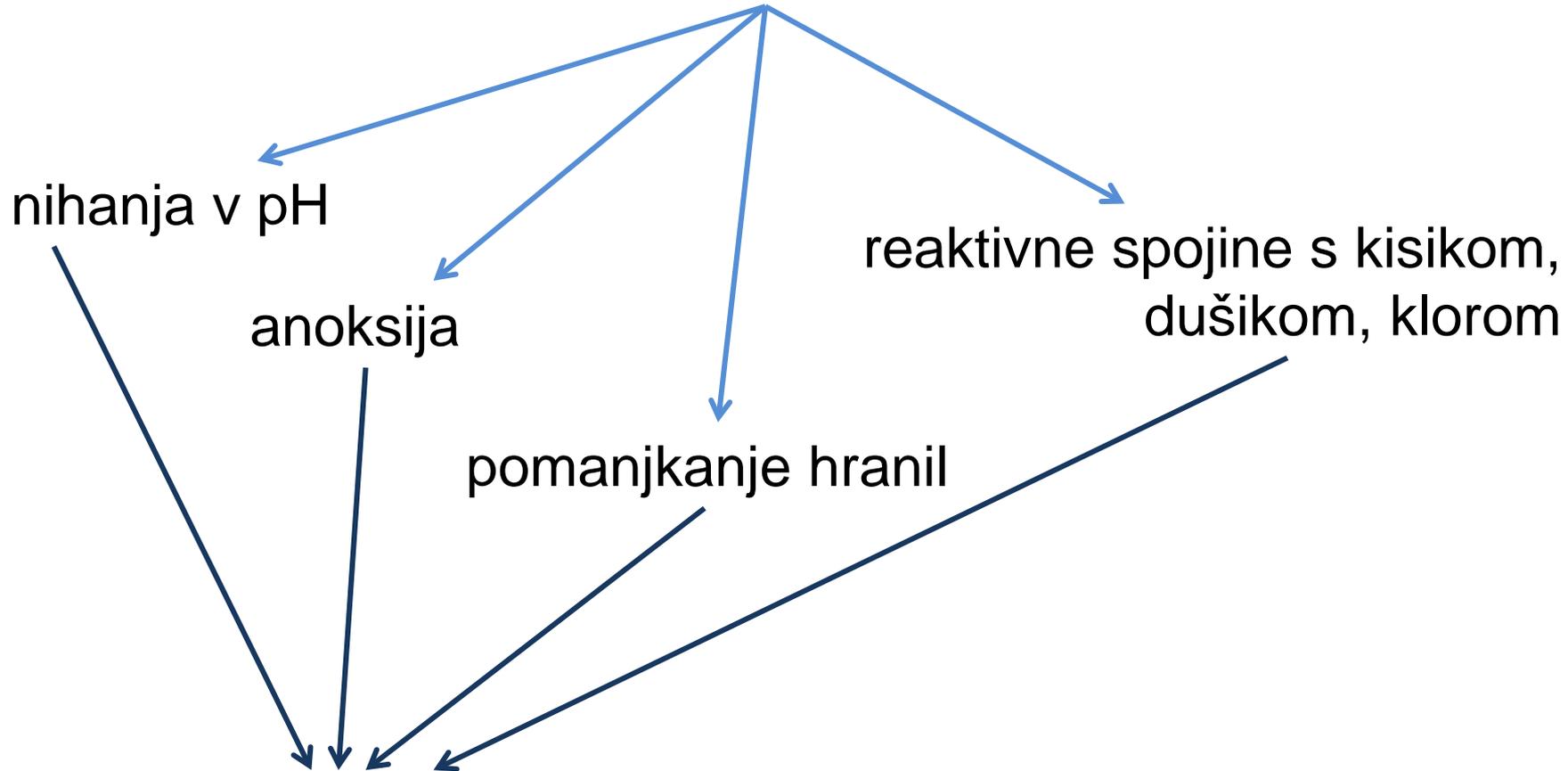
Toleranca na stres je pomembna za patogenezo:

# **ALI SO POLIEKSTREMOTOLERANTNE GLIVE PATOGENE?**

# Ubikvitarnost.



*Cryptococcus neoformans* se med infekcijo sooča s številnimi stresnimi dejavniki.

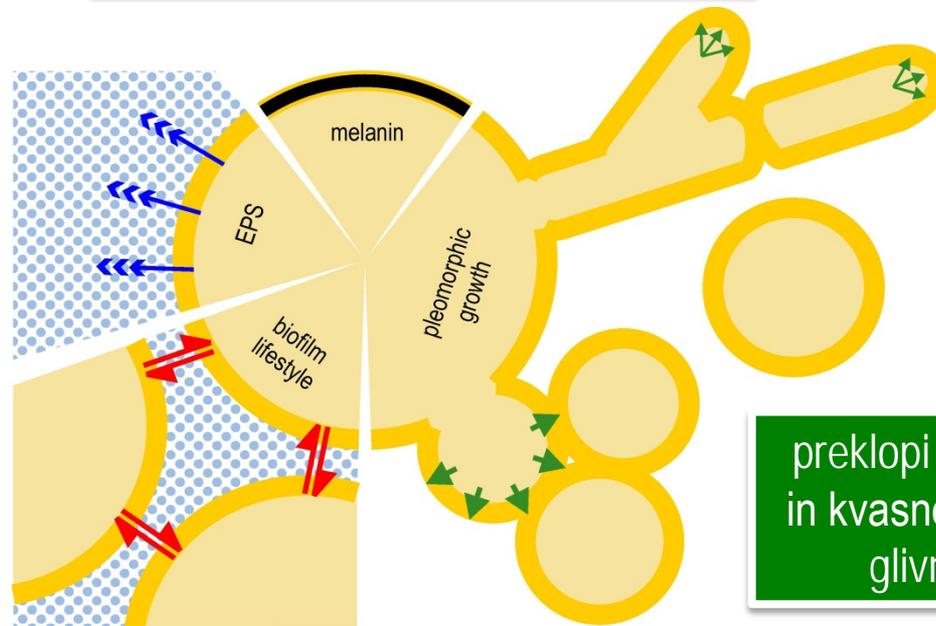


Celični odgovori na te stresne dejavnike so se verjetno razvili zaradi stresa v okolju in ne med infekcijo.

# Splošne prilagoditve poliekstremotolerantnih gliv imajo vlogo v patogenezi.

zaščita pred antimikotiki  
lovljenje reaktivnih spojin s kisikom  
oviranje fagocitoze

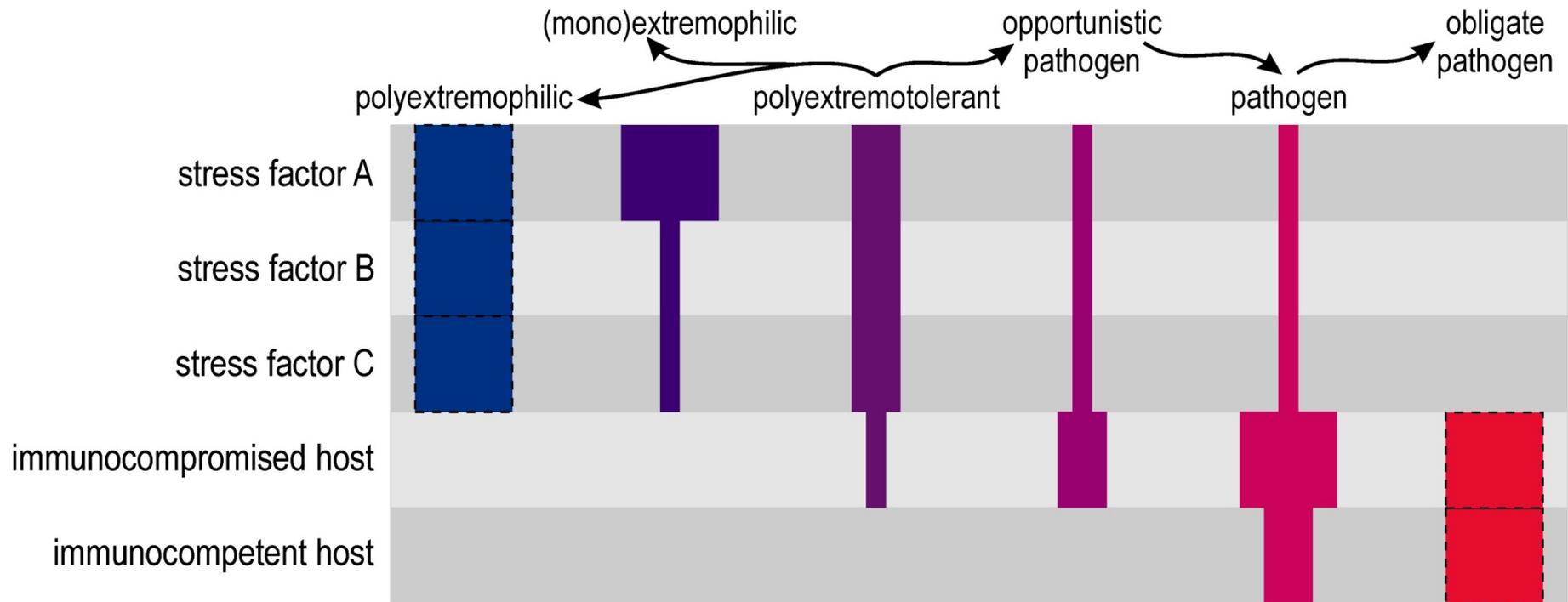
zaščita pred stresom  
dejavnik virulence



preklopi med filamentozno  
in kvasno rastjo: značilnost  
glivnih patogenov

zaščita pred imunskim sistemom  
zaščita pred antimikotiki

# Poliekstremofili kot rezervar za evolucijo ekstremofilov in (živalskih) patogenov?



# Nove glivne patogene pogosto najdemo v domačih okoljih: primer *Exophiala dermatitidis*.



Zalar P., Novak M., de Hoog G.S., Gunde-Cimerman N. Dishwashers: man-made enrichment machinery for human opportunistic fungi? Fungal Biology.



# Poliekstremotolerantne glive: oportunistične in ne patogene.

Klinična mikologija postaja izjemno zahtevno raziskovanje okužb, ki jih povzroča cela vrsta taksonomsko raznolikih gliv. Sporočilo zdravnikom in kliničnim mikrobiologom je, da **popolnoma nepatogene glive ne obstajajo: vsaka gliva lahko v dovolj imunsko oslabljenem sistemu povzroči smrtonosno okužbo**, zato nobene ne smemo zanemariti kot naključno kontaminacijo.

M. A. Pfaller, and D. J. Diekema: **Rare and Emerging Opportunistic Fungal Pathogens: Concern for Resistance beyond *Candida albicans* and *Aspergillus fumigatus***. J Clin Microbiol. 2004 October; 42(10): 4419–4431.



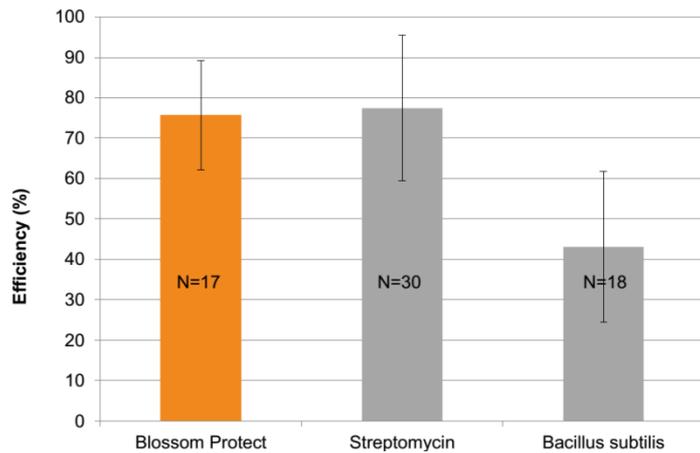
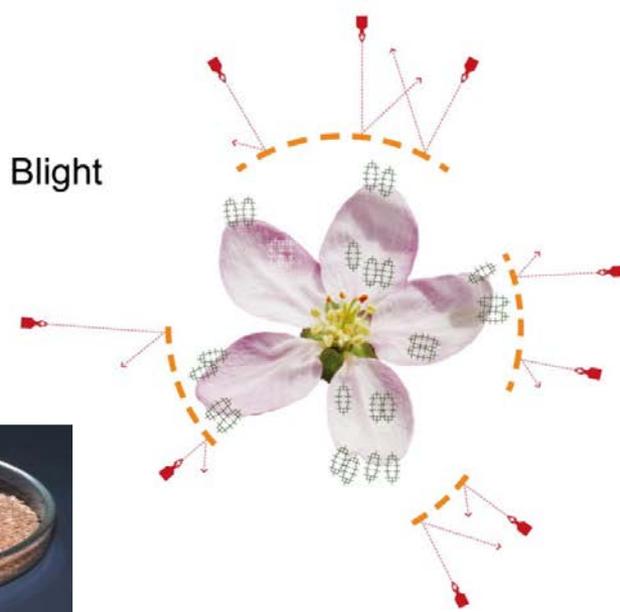
Seed culture

Liquid fermentation

Fluidized bed dryer

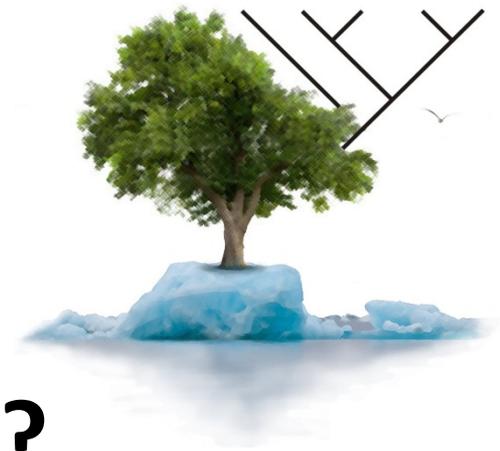
Granules (WG)

# Biotechnological Bactericide against Fire Blight The alternative to antibiotics



Poliekstremotolerantne glive:

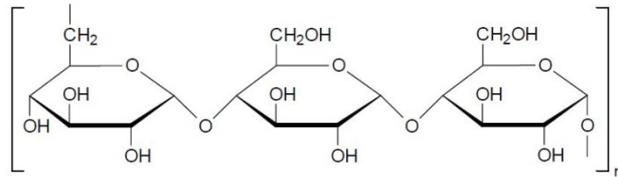
**ALI JIH LAHKO  
KORISTNO UPORABIMO?**



# A. pullulans: rešitev za vse težave.

pululan

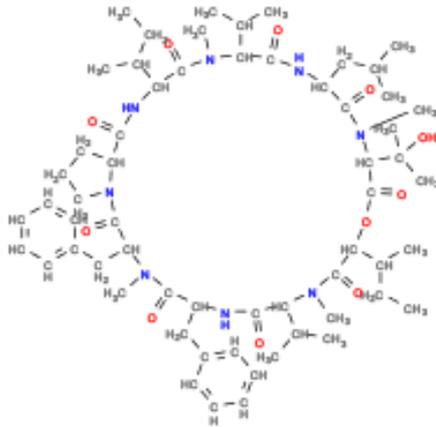
beta glukan



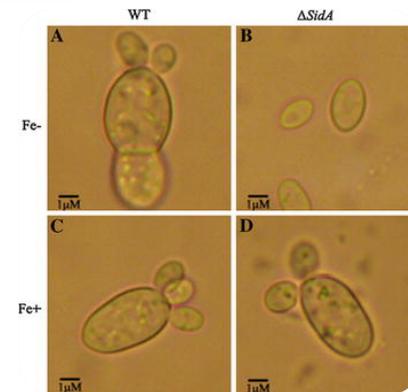
zunajcelični encimi

amilaze, celulaze, lipaze,  
proteaze, ksilanaze,  $\beta$ -  
fruktofuranozidaze,  
maltoziltransferaze, mananaze,  
lakaze

Aureobasidin A



Chi Z, Wang XX, Ma ZC, Buzdar MA, Chi ZM: **The unique role of siderophore in marine-derived *Aureobasidium pullulans* HN6.2.** *Biometals* 2012, **25**:219-230.  
Takesako K, Ikai K, Haruna F, Endo M, Shimanaka K, Sono E, Nakamura T, Kato I, Yamaguchi H: **Aureobasidins, New Antifungal Antibiotics Taxonomy, Fermentation, Isolation, and Properties.** *J Antibiot (Tokyo)* 1991, **44**:919-924.

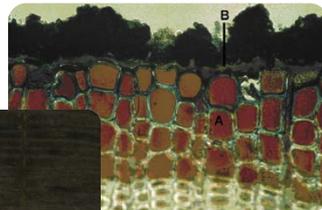


siderofori

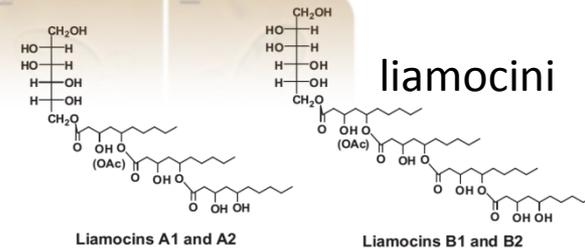
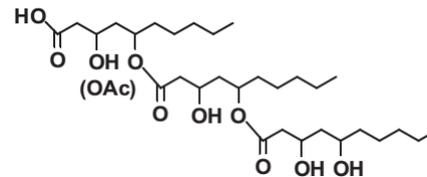
biološki nadzor

rastlinskih patogenov

zaščita lesa



exophilin A

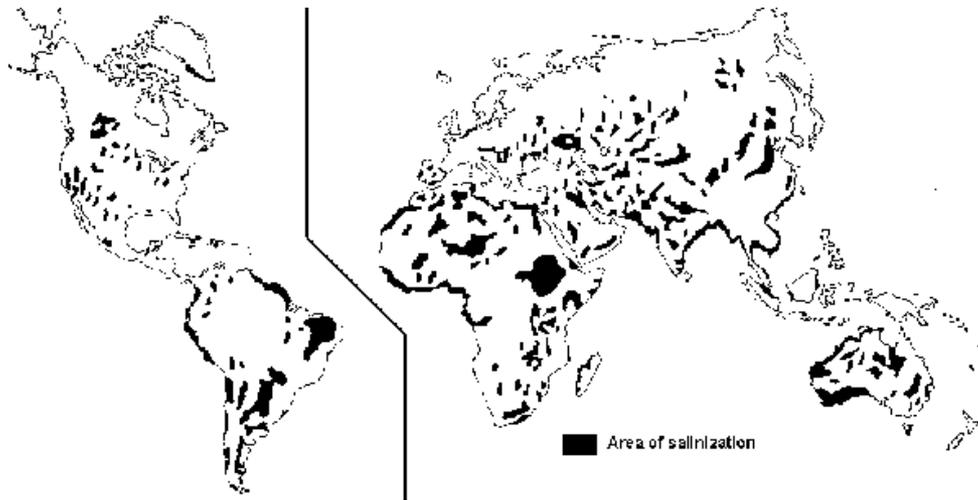


liamocini

Sailer MF, van Nieuwenhuijzen EJ, Knol W: **Forming of a functional biofilm on wood surfaces.** *Ecol Eng* 2010, **36**:163-167.

Price NPJ, Manitchopisit P, Vermillion KE, Bowman MJ, Leathers TD: **Structural characterization of novel extracellular liamocins (mannitol oils) produced by *Aureobasidium pullulans* strain NRRL 50380.** *Carbohydr Res* 2013, **370**:24-32.

Zasoljevanje je težava na tretjini namakalnih površin in zmanjšuje namakalne površine za 1-2% na leto (FAO, 2002; Lakhdar et al., 2009, Tóth et al., 2008).



Szabolcs I. 1985. Salt affected soils as a world problem. Proc. Internat. Symp. Reclamation of Salt-affected Soils I. Jinan, China. 13-21 May 1985.

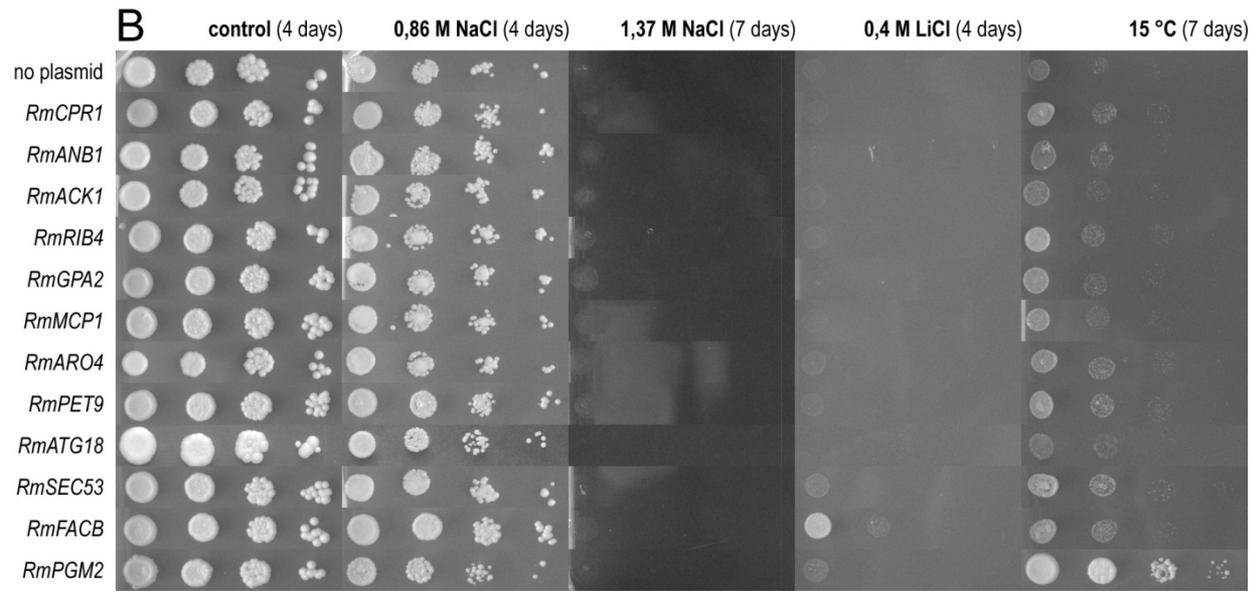
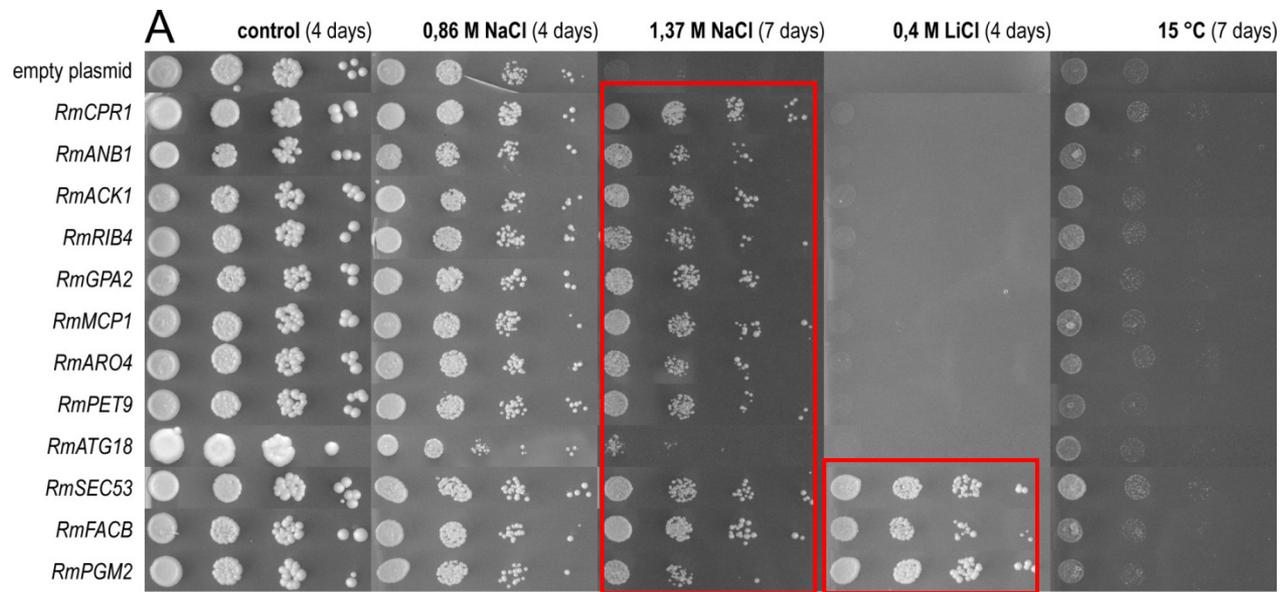


Jelte Rozema and Timothy Flowers. 2008. Crops for a Salinized World. Science: Vol. 322. no. 5907, pp. 1478 - 1480

Nizka vodna aktivnost je težava tudi v industrijski mikrobiologiji (npr. ob proizvodnji bioetanola).

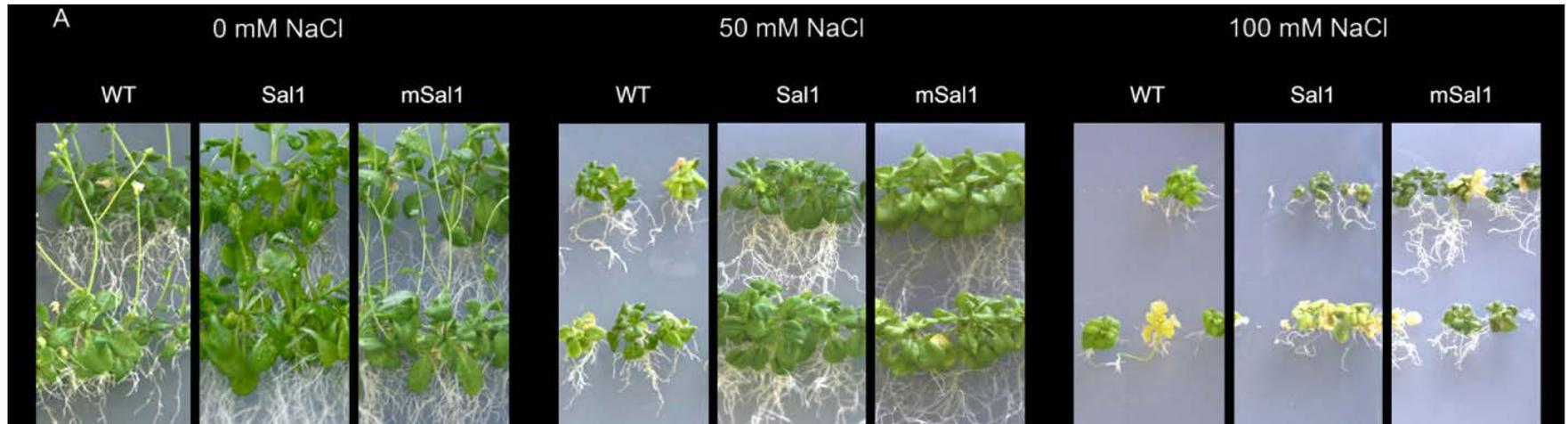


Z geni iz poliekstremotolerantnih kvasovk lahko povečamo toleranco na sol pri *Saccharomyces cerevisiae*



**A** Transformante, ki izražajo navedene transgene.  
**B** Transformante po odstranitvi plazmida s transgenom.

# Izboljšamo pa lahko tudi rast rastlin pri povišanih koncentracijah soli.



	<b>META</b>		
ApHa12	GCPNLPID	<b>DSEPLTEDL</b>	GANASDAEGK - GVLMSAILGKGADSRPLTRGALKNATTISMKR 225
HwHa12A	GCPNLPVS	<b>DSEPLREGI</b>	GADASDEEGKFGVLFSAVQNQGAQSRPLSKAGLTQPHPINMKP 226
Ha12	GCPNLVLSS	-----	YGAQDLKGHESFGYIFRAVRGLGAFYSPSS - -DAESWTKIHVRH 224
	*****	:..	** : . . . * :: * : . ** * : . * ::

Buh Gašparič M, Lenassi M, Gostinčar C, Rotter A, Plemenitaš A, Gunde-Cimerman N, Gruden K, Zel J: Insertion of a Specific Fungal 3'-phosphoadenosine-5'-phosphatase Motif into a Plant Homologue Improves Halotolerance and Drought Tolerance of Plants. Plos One 2013, 8.







# In številni drugi sodelavci ...

**Ana Plemenitaš, Metka Lenassi in sod.**

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**Martin Grube**

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**Jens Frisvad**

Technical University of Denmark, Lyngby, Denmark

**Aharon Oren**

Hebrew University of Jerusalem, Izrael

**Anna A. Gorbushina**

Carl-von-Ossietzky University, Oldenburg, Nemčija

**Erwin A. Galinski**

Institute for microbiology and biotechnology, Bonn University, Nemčija

**José Ramos**

University of Córdoba, Španija

...

Genomes: Canada's Michael Smith Genome Sciences Centre (Canada), BGI (PR China), JGI (USA)